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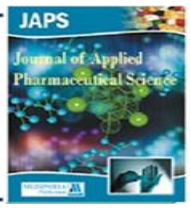

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Screening of antimicrobial activity of herbal extract of *Embelia Basal*, Chlorhexidine & Amoxicillin against salivary microflora of mixed dentition age group

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ABSTRACT

The word, Ayurveda in Sanskrit roughly means the "science or knowledge of life." Other Ayurvedic experts however, contend that Ayurveda is even more accurately translated as the "science or knowledge of longevity." As such, it focuses on comprehensively addressing the body and preventing disease by reestablishing equilibrium. When balance is achieved, longevity – living a long, healthy, active and productive life – can be obtained(Frawley D.1989., Svoboda RE 1989). Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in the different countries and are a source of many potent and powerful drugs(Srivastava J *et al*., 1996). In this study the Antimicrobial activity of 'Embelia Basal' in acetone extracts were compared with Chlorhexidine and Amoxicillin 125mg and Amoxicillin 250mg against human salivary microfloraat different concentrations. The antimicrobial activity was assisted by measuring the inhibition zones by well diffusion method. Saliva was collected from children of age group 6-12 years having DMFT value four or above four. Ten salivary samples were tested for antimicrobial property to determine the Minimum Inhibition Concentration in order to increase the reliability and precision of the study. The results confirmed the antimicrobial potential of 'Embeiia Basal' plant at different concentrations in acetone extracts are comparable with chlorhexidine and Amoxicillin and can be used as preventive and therapeutic measure in dentistry.

Keywords: *Embelia Basal*, Chlorhexidine, Amoxicillin.

INTRODUCTION

Dental decay, also known as dental caries, is defined as a disease of the hard tissues of the teeth caused by the action of microorganisms, found in plaque, on fermentable carbohydrates (principally sugars). At the individual level, caries is a preventable disease. Given its dynamic nature the disease, once established, can be arrested or reversed prior to significant cavitation taking place(Kidd AM J-B2005). Primary prevention among adolescents is aparticularly important issue in India, due to high population numbers and wide economic, social and health disparities among its population. In 2000, approximately 30% of India's population was aged 10 to 24 year, that increased to 53% when children younger than 10 were included(U.S. Census Bureau 2003).

Many efforts have been made to discover new antimicrobial compounds from various kinds of sources such as microorganisms, animals and plants. One of such resources is folk medicines. Systematic screening of them may result in the discovery of novel effective compounds (Tomoko *et al*., 2000). The increasing prevalence of multidrug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics raises the specter of untreatable bacterial infections and adds urgency to the search for new infection fighting strategies (Sieradsxi *et al*., 1999). Plants and herbs have attained a significantrole not only as therapeutic agent but also as health maintaining agent. The genus Embelia has been investigated for a variety of purposes in Ayurveda(V. S. Agarwal 1997). It is a shrub from family 'Myrsinaceae', an Indian variety, is widely distributed throughout India and commonly known as 'Vidanga'. The larger elliptical leaves of the plants are used in combination with ginger, are used as a gargle for sore throats. The dried bark of the root is used as a remedy for toothache and the finely powered berries are formulated as an ointment for treating pleurities. E. basal is highly esteemed in Ayurvedic medicine with powerful anthelmintic and antioxident properties and also an important constituent of number of formulations(Gayatri S. Kamble *et.al*, 2011).E.basal shows significant anti-microbial property(Gayatri S. Kamble *et.al*,2011).Although Embeliaribes has been studied for anti-cariogenic properties(Tsuneo Namba 1985).In this study we are investigating the antimicrobial properties of 'Embelia Basal' in acetone extract with chlorhexidine and Amoxicillin125mg and Amoxicillin250mg at increasing concentration.

MATERIALS AND METHODS

Plant Material

The fruits of Embelia basal (R & S) A. Dc. Family Myrsinaceae are obtained as a market sample. The fruits are authenticated by Agharkar Research Institute, Pune, Maharashtra, India. Its voucher specimen No. is AHMA F-084. Preparation of acetone Extract. Air shade dried and powdered fruit material (10 gm) was refluxed with acetone for 18 hours. The yield of Acetone extract was found to be 11.6%. This extract was further used for experiments. Sample of each acetone extract (50 mg) were dissolved in respective solvents (5 ml). The well (8mm) was filled with these extract of different concentrations ranging from 50µg to 800µg per well.

Criteria for selection of patients

In the present study, patients of 6-12 years of age, in mixed dentition period with DMFT value four or more were included. These patients had no history of antibiotic therapy or use of chemical anti-plaque agents prior to six months of study initiation.

Method of saliva collection and storage

The subjects were told to rinse with water; saliva was allowed to accumulate in the floor of the mouth for approximately two minutes and by asking the subject to spit in the funnel, saliva (3ml) was collected in a vial . By following the above mentioned method, 10 samples were collected in the early morning time. These salivary samples were diluted (3:1 ratio) in the sterile vials containing 1ml of normal saline and were used to inoculate on the agar plates. All samples were refrigerated within 30 minutes and frozen within 4 hours.

Antimicrobial Assay

The microbial inhibition assay was prepared using the agar well diffusion method. Sterile 8.0mm diameter of well were impregnated with the extract of different concentrations ranging from 50µg to 800µg per well. Adequate amount of Muller Hinton Agar were dispensed into sterile plates and allow solidifying under aseptic conditions. The test samples of saliva (0.1ml) were inoculated with a sterile spreader on the surface of solid Muller Hinton Agar medium in plates. After the media was solidified; a well was made in the plates with the help of a cup-borer (8.0mm). The well was filled with different concentrations of the extract (50µg to 800µg/ well) and plates were incubated at $37 \pm 0.1^{o}C$ for 24 hours. After incubation, the plates were observed for zones of growth of inhibition and the diameters of these zones were measured in millimetres by using bacterial inhibition zone reading scale. All the tests were performed under sterile conditions. Chlorhexidine was used as positive control. The lowest dose required to attain maximum inhibition of a mixed oral micro flora was recorded. The dose dependent maximum inhibition zones of a mixed oral micro flora was recorded.

RESULTS AND DISCUSSION

This study compares antimicrobial activity of embelia basal with 0.2% chlorhexidine, amoxicillin 125mg and amoxicillin 250mg. The zone of inhibition are measured by excluding the diameter of well. The mean value of average zone of inhibition of embelia basal, chlorhexidine and amoxicillin 125mg and amoxicillin 250mg in ten salivary samples has taken for comparison. These zones of inhibition are directly proportional to the concentration.

Table. 1: Mean value of Zones of inhibition of acetone extract of embelia basal in salivary samples.

Acetone extract of Embelia Basal	Mean value of average zone of inhibition
50 mg	4.1000
100 mg	6.4000
200 mg	9.5000
400 mg	11.0000
800 mg	13.2000

Table 2: Mean value of Zones of inhibition of standard antimicrobial agent in salivary samples.

Antimicrobial agent	Mean value of average zone of inhibition
0.2% chlorhexidine	20.0000
Amoxicillin 125mg	40.4000
Amoxicillin 250mg	48.4000

Table 1 represents the mean value of average zone of inhibition of the acetone extract of *Embelia basal* at five

different concentrations. Table 2 represents the mean value of average zone of inhibition of chlorhexidine and amoxicillin 125mg and amoxicillin 250mg in ten salivary samples. Results were obtained after 24 hours of incubation. It must be noted that the acetone extract had produced zones of inhibition 20mm, 22mm and 28mm for concentrations 200µg , 400µg , and 800µg respectively only in the fifth saliva sample which could be a false positive result. Number 1, 3 and 4 represents zone of inhibition of chlorhexidine and amoxicillin 125mg and amoxicillin 250mg respectively (fi1). This study proves that the antimicrobial activity of Embelia Basal at higher concentration is comparable with 0.2% chlorhexidine. But to compare the antimicrobial activity of Embelia Basal with amoxicillin we need to investigate further higher concentration of extracts. Statistically, Kruskal-Wallis test followed by post-hoc test proved that all results are comparable as the p value is 0.0001 which is significant ($p < 0.5$). To prove antimicrobial activity of Embelia Basal with amoxicillin we need to take further higher concentration. The use of antimicrobials has increased steadily since the discovery of penicillin(PovedaRoda.,et al 1985). Numerous drugs have been developed since then, few of which were considered potentially toxic(6 D'Arcy PF 1994). Nature always stands as a golden mark to exemplify the outstanding phenomena of symbiosis. Natural products from plant, animal and minerals have been the basis of the treatment of human disease. Today estimate that about 80 % of people in developing countries still relays on traditional medicine based largely on species of plants and animals for their primary health care. Herbal medicines are currently in demand and their popularity is increasing day by day. Medicinal plants play an important role in the development of potent therapeutic agents(SheetalVerma et al). Therefore the use of Aurvedic medicines has increased now a days. This study compares the antimicrobial properties obtained by a plant and which is easily available to the common man. It may have fewer side effects as it falls in the category of natural medicine. The Acetone extract of Embelia Basal can be formulated in the form of a mouth wash or as an intracanal medicament where an antimicrobial agent is required.

Fig 1: Here '1', '2' and '3' represents zone of inhibition of standard antimicrobial agent 0.2% Chlorhexidine, amoxicillin 125mg and amoxicillin 250mg respectively.

Fig. 2: number 1to5 represents Different concentrations of acetone extract from 50µg,100µg,200µg,400µg,800µg and different zones of inhibiton respectively.

CONCLUSION

The antimicrobial activity of embelia basal at higher concentration is comparable with 0.2% chlorhexidine. This study has confirmed the antimicrobial potentials of the plant, thus supporting its application as a preventive remedy for various microbial diseases of hard tissues in the oral cavity.

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